REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Detection of DNA Sequence Variation. There is a great need in both basic and clinical research to identify DNA sequence variations with high efficiency and accuracy. The current techniques for detection of such variation can be divided into two groups: 1) detection of known mutations or polymorphisms and 2) detection of unknown mutations or polymorphisms (also referred to as mutation scanning). A variety of effective methods have been developed for detecting known mutations and polymorphisms and include techniques such as direct DNA sequencing, allele-specific oligonucleotide hybridization, allele-specific PCR, DNA arrays, and PCR/LDR. There are a variety of techniques for detecting unknown mutations, but their sensitivity and accuracy vary greatly.

Comparison of High-Throughput Techniques to Identify Unknown Mutations in Clinical Samples. Identifying unknown mutations in clinical samples presents similar difficulties as screening for known mutations, as well as some novel complications. A mutation present in a tumor sample may represent as little as 15% of the DNA sequence for that gene due to stromal contamination. Therefore, screens for unknown mutations require high sensitivity in order to identify low abundance sequence. Since most cancer genes contain multiple exons which may be altered, even for commonly mutated genes (e.g. PTEN), most assay results of a single exon will be negative. However, by pooling samples together, the probability of finding a significant mutation in a given assay increases. In order to increase the capacity of a screen by pooling samples, the technique must have a high enough sensitivity to tolerate further mutation dilution which results from pooling. Further, for uncommon germline mutations, the ability to pool samples greatly improves the throughput of evaluating large numbers of samples in multiple exons.

Other significant complications associated with screening for unknown cancer mutations are the need to: (i) Identify either frameshift, nonsense, or missense mutations, and (ii) distinguish missense mutations from germline (i.e., silent) polymorphisms. The latter is of great significance, because it is estimated that polymorphisms exist approximately once every kb in the human genome. Separating out the apparently less interesting polymorphisms should significantly increase the efficiency in identifying informative mutations. Unfortunately, present methods of identifying additional low frequency mutations with clinical significance are restricted in their applications. Most methods to date lack either the

accuracy to discriminate or the sensitivity to be an efficient technique. As a result, there is an urgent need for a scanning method with the potential to identify precise mutations and with the sensitivity to analyze tumors or germline DNA in pooled samples.

Direct Sequencing and Variation Detection Arrays. A variety of methods have been developed to scan for unknown mutations. Direct sequencing represents an ideal in that it can detect any mutation and its position, although high throughput is costly. This method has low sensitivity which prevents an accurate analysis of pooled samples, and, in regard to solid tumor samples, the technique is vulnerable to stromal contamination. In blinded studies, direct sequencing was unable to identify 20% of K-*ras* mutations in microdissected tumor samples. This number is consistent with other studies in which 24% of p53 mutations in lung tumor samples were not identified. These results reflect the limits of sensitivity associated with direct sequencing and further demonstrate the difficulty of automating this approach. If a sample is known to have a mutation in a specific area, then repeated attempts of direct sequencing should be able to identify the mutation and its position. Therefore, direct sequencing may have utility as a second step to identify the exact base changed in a gene region previously identified as containing a mutation.

Variation detection arrays (VDA) use standard hybridization microarrays to scan large sequence blocks in given genes. Despite the high scanning capacity levels, this approach has some characteristics that limit its utility. VDA is unable to detect all mutations and has particular difficulty in detecting frameshift mutations, for example in the BRCA2 and p53 genes. High false positive rates of 11-21% have been observed using VDA. One consequence of direct hybridization that may account for these inaccurate results involves the disruption of secondary structure. A perfect match PCR fragment may assume a secondary structure that is not present in variant fragments. Since a secondary structure is usually energetically unfavorable with respect to fragment/array hybridizations, the variant fragment may bind the perfect match complement on the array with higher binding affinity than the true perfect match fragment. Such illegitimate hybridizations would produce a false positive signal. Direct hybridization of mutation-containing PCR fragments to sequences on the array has the additional difficulty of simultaneously assaying sequence tracts with localized regions of both high G+C and A+T content. Certain mutations within these tracts can significantly decrease the Tm and thus lead to false negative signals.

Gel-Based Assays, Mismatch Cleavage Enzymes, and Protein Truncation Assays. Other methods that are widely used to detect unknown mutations resolve homoduplex and heteroduplex DNA based on their differing electrophoretic migration

behavior. These methods include single-stranded conformational polymorphism (SSCP), denaturing-gradient gel electrophoresis (DGGE), constant denaturing capillary electrophoresis (CDCE), dideoxy fingerprinting (ddF), and restriction endonuclease fingerprinting (REF). A similar approach, denaturing high-performance liquid chromatography (DHPLC), also resolves homoduplex from heteroduplex DNA but is based on separation by ion-pair reverse-phase liquid chromatography on alkylated nonporous (styrene divinylbenzene) particles. Although these techniques contain some very desirable characteristics, none of them are complete with respect to both previously-discussed throughput and sensitivity. The techniques which can identify the position of the polymorphism (ddF and REF), are not applicable for evaluating low level mutations in pooled samples. The rest of these techniques tend to be rapid and can detect low level mutations, but they cannot distinguish missense from silent polymorphisms. In addition, since these methods do not locate the position of the mutation, they are less compatible with follow up techniques such as direct sequencing.

A sophisticated approach for detecting frame-shifts or termination codons in the APC gene uses a coupled transcription translation assay referred to as the protein truncation test. This is currently the most robust approach for finding mutations which generate truncated proteins in large genes; however, it does not detect missense mutations or polymorphisms. Polymorphisms may also be identified by cleavage of mismatches in DNA hybrids, such as DNA-RNA heteroduplexes via RNase A mismatch cleavage, as well as in DNA-DNA homoduplexes via chemical mismatch cleavage (CCM), T4 Endonuclease VII or MutY cleavage or via cleavase. Recently, a plant endonuclease, CEL I, with similar activity to T4 endonuclease VII has been described. CEL I has similar activity to nuclease S1 and works at neutral pH. Its cleavage efficiency and background varies according to the mismatch and specific template examined, and further evaluation of other templates (e.g. in GC-rich regions) is still required. The most accepted mismatch cleavage approaches identify the approximate position of the polymorphism by using T4 Endonuclease VII or MutY to cleave a heteroduplex of normal and polymorphic substrate at a mismatch. These enzymatic cleavage approaches identify the approximate position of most polymorphisms; however, these enzymes often nick matched DNA causing a high background noise. This high background tends to limit their usefulness with respect to solid tumor studies.

The present invention is directed to overcoming the above deficiencies in the art.

The rejection of claims 1-41, 46-86, and 155 under 35 U.S.C. 112 (2nd para.) for indefiniteness based on the recitation of "can" in claims 1, 46, and 155 is respectfully traversed in view of the above amendments.

The rejection of claims 1-41, 46-86, and 155 under 35 U.S.C. 112 (2nd para.) for indefiniteness based on the recitation of "substantially" in claims 1, 17, 46, 62, and 155 is respectfully traversed.

Applicants submit that use of the term "substantially" in patent claims is well accepted and, that the meaning of "substantially" as recited in the claims would have been clear to one of ordinary skill in the art. The Federal Circuit has repeatedly held that "substantially" is not indefinite, rather, it "is a descriptive term commonly used in patent claims to 'avoid a strict numerical boundary to the specified parameter.'" *See e.g., Ecolab, Inc. v. Envirochem, Inc.*, 264 F.3d 1358, 1367, 60 U.S.P.Q.2d 1173, 1179 (Fed. Cir. 2001), citing *Pall Corp. v. Micron Seps.*, 66 F.3d 1211, 1217, 36 U.S.P.Q.2d 1225, 1229 (Fed. Cir. 1995) and *Andrew Corp. v. Gabriel Elecs. Inc.*, 847 F.2d 819, 821-22, 6 U.S.P.Q.2d 2010, 2013 (Fed. Cir. 1988).

Accordingly, the phrase "substantially no resealing of nicked heteroduplexed products at locations adjacent to mismatched base pairs" in claims 1, 46, and 155 and the phrase "substantially no KCl or NaCl" in claims 17 and 62 cannot be properly rejected due to the presence of the word "substantially". Accordingly, the rejection of claims 1-41, 46-86, and 155 for indefiniteness based on the recitation of "substantially" is improper and should be withdrawn.

The rejection of claim 37 under 35 U.S.C. 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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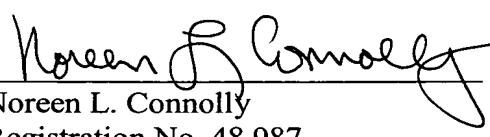
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